Notice of Allowability	Application No.	Applicant(s)	
	10/089,040	UMEZAWA ET AL.	
	Examiner	Art Unit	
	William W. Moore	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *the amendment filed 8 June 2006 and the interview conducted 7 September 2006*.

2. ☒ The allowed claim(s) is/are *1,3,9,10 and 13-15*.

3. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☐ Information Disclosure Statements (PTO-1449 or PTO/SB/08), Paper No./Mail Date _____
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application (PTO-152)
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☒ Examiner's Amendment/Comment
8. ☒ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Amend claims 1, 3, 9, 10, 12 and 13 thus:

1. (Amended) A set of probes for analyzing protein A - protein B interactions, which set comprises:

(i) probe "a" comprising a fusion polypeptide wherein the carboxyl terminus of ~~comprising an N-terminal portion of an intein polypeptide and~~ an N-terminal portion of an indicator protein~~, wherein the N-terminal portion of the indicator protein~~ is fused to the N-terminus of the N-terminal portion of an ~~the~~ intein polypeptide, and wherein the C-terminus of the intein polypeptide is fused to ~~capable of fusing with~~ a target protein A; and

(ii) probe "b" comprising a fusion polypeptide wherein the amino terminus of ~~comprising a C-terminal portion of the intein polypeptide and~~ a C-terminal portion of the indicator protein~~, wherein the C-terminal portion of the indicator protein~~ is fused to the C-terminus of the C-terminal portion of the intein polypeptide, and wherein the N-terminus of the intein polypeptide is fused to ~~capable of fusing with~~ a target protein B,

wherein the N-terminal portion of the intein and the C-terminal portion of the intein are sufficient to effect trans-splicing, and

wherein the N-terminal portion of the indicator protein and the C-terminal portion of the indicator protein constitute a functioning indicator protein after intein mediated trans-splicing occurs.

3. (Amended) The set of probes for analyzing protein A - protein B interaction ~~analysis~~ of claim 1, wherein the C-terminus of the intein polypeptide ~~C-terminal~~ of probe "a" and the N-terminus of the intein polypeptide ~~N-terminal~~ of probe "b" each further comprise ~~contain~~ a peptide linker sequence.

9. (Amended) The set of probes for analyzing protein A - protein B interaction of claim 14 ~~1~~ wherein the luminescent enzyme is a luciferase.

10. (Amended) A method for analyzing protein A - protein B interaction by using the set of probes of claim 1, which method comprises:

(i) preparing a recombinant polynucleotide encoding the fused protein of probe "a" under the control of a promoter,

(ii) preparing a recombinant polynucleotide encoding the fused protein of probe "b" under the control of a promoter

(iii) transforming an eukaryotic host cell with the recombinant polynucleotides of (1) and (2) and culturing the cell under conditions permitting the expression of the fused proteins encoded by (i) and (ii), so that intein-mediated trans-splicing may occur,

~~fusing the C-terminus of the intein polypeptide of probe "a" with a target protein A, and fusing the N-terminus of the intein polypeptide of probe "b" with a target protein B;~~

~~introducing probe "a" and probe "b" in a system under conditions permitting excision of the intein portions of probes "a" and "b" upon interaction of proteins A and B;~~ and

(iv) detecting the interaction of protein A with protein B by measuring a change of a signal from the indicator protein resulting from intein-mediated trans-splicing and that consists ~~consisting~~ of the N-terminus of the indicator protein and the C-terminus of the indicator protein,

whereby the interaction of protein A and protein B is analyzed.

12. (Amended) A vector for expressing a set of probes for analyzing protein A - protein B interaction, which vector co-expresses two probes wherein probe "a" is ~~comprising~~ a fusion polypeptide comprising ~~of~~ an N-terminal portion of an intein polypeptide and an N-terminal portion of an indicator protein, and probe "b" is ~~comprising~~ a fusion polypeptide comprising ~~of~~ a C-terminal portion of the intein polypeptide and a C-terminal portion of the indicator protein, wherein the vector comprises:

(1) a polynucleotide encoding the fusion polypeptide of probe "a" wherein the coding region for the N-terminal portion of the indicator protein is ligated at the 5' side of the coding region for the N-terminal portion of the intein polypeptide, and a 3' side of the coding region for the N-terminal portion of the intein polypeptide is a cloning site for ligating a ~~the~~ polynucleotide encoding protein A; and,

(ii) a polynucleotide encoding the fusion polypeptide of probe "b" wherein the coding region for the C-terminal portion of the indicator protein is ligated at the 3' side of the region for the C-terminal portion of the intein polypeptide, and a 5' side of the coding region for the C-terminal portion of the intein polypeptide is a cloning site for ligating a ~~the~~ polynucleotide encoding protein B;~~,~~

wherein the N-terminal portion of the intein and the C-terminal portion of the intein are sufficient to effect trans-splicing, and

wherein the N-terminal portion of the indicator protein and the C-terminal portion of the indicator protein constitute a functioning indicator protein after intein mediated trans-splicing occurs.

13. (Amended) A method for analyzing protein A - protein B interaction by using the expression vector of claim 12, which comprises:

(i) ligating a ~~the~~ polynucleotide encoding protein A into the expression vector at ~~to~~ the 3' side of the coding region for the N-terminal portion of the intein polypeptide and ligating a ~~the~~ polynucleotide encoding protein B into the expression vector at ~~to~~ the 5' side of the coding region for the C-terminal portion of the intein polypeptide;
(ii) transforming an eukaryotic host cell with ~~introducing~~ the vector of step (1) ~~into a eukaryotic cell~~ and ~~thereby~~ expressing probe "a" ~~fusing the C-terminus of the intein polypeptide to protein A in the eukaryotic cell~~ and ~~expressing~~ probe "b" ~~fusing the N-terminus of the intein polypeptide to protein B~~ in the eukaryotic host cell~~,~~ under conditions permitting excision of the intein portions of probes "a" and "b" upon the interaction of proteins A and B; and
(3) detecting the interaction of protein A with protein B by measuring a change of a signal from the indicator protein that is a fusion protein of the N-terminus of the indicator protein and the C-terminus of the indicator protein,
whereby the interaction of protein A and protein B is analyzed.

Add the new claims 14 and 15:

14. (New) The set of probes according to claim 1 wherein the indicator protein is a green-fluorescent protein or a luminescent enzyme.

15. (New) The vector of claim 12 wherein the indicator protein is a green-fluorescent protein or a luminescent enzyme.

Authorization for this examiner's amendment was given in a telephone interview with Mr. Jay F. Williams on 7 September 2006.

The following is an examiner's statement of reasons for allowance: The examiner's amendment above clarifies the descriptions of certain elements of the claims, removes redundancies, and conforms the recitations of claim 1 and 10 with the Drawing Figures 1 and 4-11 and disclosures at pages 10-21 of the substitute specification filed 2

December 2005. The subject matter of the claims is free of the prior art of record herein because there is no suggestion in the prior art that the availability of split intein systems for *in vitro trans*-splicing of a recombinantly-expressed N-terminal polypeptide to a recombinantly-expressed, or synthetically-produced, C-terminal polypeptide could, or should, be adapted for the *in vivo* reconstitution of an enzymatic activity that would serve as a reporter, or indicator, of protein-protein interaction in a host cell. The allowed claims describe a significant advance beyond the teaching of Remy et al., of record, where non-covalent association of two fragments of a murine dihydrofolate reductase [DHFR] allowed fluorescent detection of protein-protein interaction within a mammalian host cell when a fluorescein-labelled methotrexate substrate bound to the transiently-associated DHFR fragments. Indeed, the probes, methods, and vectors of claims 1, 3, 9, 10, 12 and 13 allowed herewith permit the covalent reconstitution of any available indicator protein that provides a quantifiable signal that persists after the association of proteins the interaction of which is to be analyzed where the prior art of record teaches other indicator proteins that are divided into N-terminal and C-terminal portions and the specification teaches the modification of indicator proteins to permit their reconstitution upon trans-splicing of a split intein of the claimed probes and vectors. The necessary relationship of the elements required for both probes A and B that permit the covalent reconstitution of an indicator protein by trans-splicing *in vivo* to produce a detectable signal of protein-protein interaction inside cells is not set forth even in US Patents and Pre-Grant Publications based on applications made of record with the communications mailed 6 October 2004 that were filed after the publication of journal articles of the co-inventors herein.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably

accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is 571.272.0933 and whose FAX number is 571.273.0933. The examiner can normally be reached Monday through Friday between 9:00AM and 5:30PM EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's Supervisory Primary Examiner, Dr. Kathleen Kerr, can be reached at 571.272.0931. The official FAX number for all communications for the organization where this application or proceeding is assigned is 571.273.8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571.272.1600.

William W. Moore
7 September 2006

NASHAAT T. NASHED PHD.
PRIMARY EXAMINER